

A Combination of Alpha Monolaurin and Probiotics as Replacement for Antibiotic Growth Promoter in Broilers

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This study evaluated the effects of a combination of alpha monolaurin (AML) and probiotics (P) as a replacement for the antibiotic growth promoter, Zinc bacitracin (ZnB), on the growth performance and gut microbiota of broilers. Two hundred forty male Cobb broilers (day-old) were randomly assigned to two dietary treatments, namely: a control group supplemented with ZnB (1000 g/t) and a treatment group supplemented with AML+P (250 g/t). Each group was replicated ten (10) times with twelve (12) broilers each. The average daily gain and average feed consumption of the broilers were comparable between the two groups, but the feed conversion ratio was superior for those supplemented with AML+P than those supplemented with ZnB. Income over feed cost was higher by 3.71% for AML+P than for the ZnB supplemented broilers. Microbiota analysis of broilers' digesta (17, 19, 24, 26, and 31 d) showed that AML+P reduced lactic acid bacteria population on day 31 and affected non-detectable population of *C. perfringens* at any of the measurement periods. Based on the results, AML+P was a satisfactory replacement for ZnB supplement in the broiler diet.

Keywords: Gastrointestinal infections, bacitracin, broiler performance, gut health, medium-chain fatty acids, probiotics.

INTRODUCTION

Antibiotic growth promoters (AGPs) have long been used at sub-therapeutic doses to enhance broiler performance and prevent gastrointestinal infections, particularly necrotic enteritis (NE) caused by *Clostridium perfringens*. However, growing concerns over antimicrobial resistance and antibiotic residues in meat have driven the search for sustainable alternatives (Miyakawa et al., 2024). The broiler industry, being the fastest-growing sector of global livestock production, plays a vital role in providing affordable animal protein and contributes significantly to the agricultural economy (Gržinić et al., 2023). With global poultry production projected to continue increasing, ensuring safe, high-quality, and sustainable poultry products has become a top priority for both producers and consumers.

NE caused by *C. perfringens*, is one of the most economically important poultry diseases that is commonly seen in 2 to 5-week-old broilers. The clinical form of the disease is characterized by a high mortality rate of up to 50%, while the subclinical form does not result in significant mortality and is only associated with mild damage to the intestine. However, this may cause significant losses through impairing the ability

of the intestine to absorb nutrients, ultimately resulting in a significant reduction in productivity (Alizadeh et al., 2021). Being one of the most widely used antibiotic growth promoters, Zinc bacitracin (ZnB) inclusion in broiler diets has been imperative, both as a control measure against NE and for improving productivity. However, the continued use of AGPs has been at the center of dialogue regarding antimicrobial resistance and the presence of antibiotic residues in meat products, which pose a threat to human health (Mehdi et al., 2018). Consequently, consumer preference has shifted towards antibiotic-free poultry products, and the poultry industry is under increasing pressure to adopt safer and more sustainable feeding strategies (Bist et al., 2024).

Medium-chain fatty acids (MCFA) have been observed to exhibit antimicrobial, antifungal, and anticoccidial activities, hence, it is suggested that these types of organic free fatty acids and natural oils can be an alternative to AGPs. Lauric acid, which is a type of MCFA that is mainly derived from coconut oil, possesses strong antimicrobial activity. According to recent reports, growth in broilers can be positively affected by MCFAs, which shows a promising approach to improving the overall health and nutrition in poultry production (Van der Sluis, 2015).

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Its alpha monoglyceride form, alpha monolaurin (AML), exerts stronger antipathogenic effect by disrupting lipid membranes. It is biologically more active in neutralizing viruses and bacteria compared with non-esterified lauric acid. In addition, AML is resistant to enzymatic breakdown and is not affected by the pH range in the gastrointestinal tract, hence is stable throughout the complete gastrointestinal tract (Szabo et al., 2023).

Probiotics improve gut integrity and nutrient absorption by competing with pathogens, enhancing enzyme activity, and reducing harmful metabolites. Moreover, several probiotic strains have demonstrated the ability to inhibit common poultry pathogens such as *Salmonella*, *Escherichia coli*, and *C. perfringens*, which are associated with poor performance and economic losses in broiler operations (Naeem & Bourassa, 2025). Notably, combining functional feed additives such as AML and probiotics may yield synergistic effects: while AML exerts direct antimicrobial activity, probiotics foster a healthy gut microbiota, creating a dual approach to disease prevention and growth promotion. This combination strategy could enhance poultry resilience against gastrointestinal diseases more effectively than either the additives alone.

Despite the growing body of research on feed additives as AGP alternatives, limited studies have explored the combined use of AML and probiotics in broiler diets. Most investigations have focused on their individual effects, yet the potential for synergistic interactions remains underexplored. Furthermore, while improvements in growth performance and gut health have been reported, there is still a need to evaluate these alternatives under practical production conditions and to assess their impact on economic efficiency. Addressing these gaps is critical, as sustainable poultry production requires strategies that are not only biologically effective but also economically viable and aligned with consumer expectations. This study, therefore, seeks to bridge this knowledge gap by generating comprehensive evidence on the effects supplementing a combination of AML and a multispecies probiotic mix, with the goal of contributing to more resilient, profitable, and antibiotic-free broiler production systems.

MATERIALS AND METHODS

Experimental design, diets, and broiler management: A total of 240 day-old Cobb broilers (all males; class A) were obtained from a BAI-accredited Cobb Hatchery facility in Tanay, Rizal, Philippines. These were randomly allocated to 2 treatments assigned in a completely randomized design (CRD), replicated 10 times, with 12 broilers each. A diet with Zinc bacitracin (ZnB, 1000 g/t) served as reference diet, and a diet with combined alpha monolaurin (AML, 125 g/t) and multispecies probiotic mix (P, *Bacillus subtilis*, *Lactobacillus spp.*, *Saccharomyces cerevisiae*, 125 g/t) served as a test diet. The broilers were brooded and grown in flat-deck broiler

cages with wire flooring. Each broiler was allotted 0.028 m² and 0.093 m² during the starting and finishing period, respectively. The cages were equipped with adequate feeding and water facilities. Brooding was done using 40-watt incandescent bulbs to supply 2.67 watts per broiler for the first ten (10) days. The broilers were fed with starter, grower, and finisher diets at 1-14, 15-24, and 25 - 32 days, respectively. Restrictive feeding was applied during the first three days, and subsequently ad libitum feeding was practiced. Fresh and clean drinking water was always provided. Strict cleanliness and sanitation in the experimental area were followed. The nutrient recommendation for Cobb (2018) was used as the basis for the nutrient and energy specification of the basal booster, starter, and finisher diets. The diets were least-cost formulated and prepared as mash using corn, rice bran, soybean meal, and coconut oil as the primary feed ingredients. The starter, grower, and finisher basal diets were mixed using a horizontal-type feed mixer and were then added with the treatment feed additives using a rotary-type feed mixer.

Sampling and measurements: The broilers were grouped and weighed by pen at the time of their arrival in the experimental area. They were also weighed by the group at 14, 24 and 32 days of age after 3 hours of feed withdrawal. The weight of dead birds was obtained and recorded, including the day death occurred. Gain in weight was calculated by subtracting the initial weight from the sum of the final weight and the weight of dead broilers. Average daily gain (ADG) in weight was computed as the quotient of gain in weight and the number of bird days. Broilers in each pen were given their pre-weighed diets; the diets left at the end of the feeding period were weighed and deducted from the amount of pre-weighed diets to determine group feed consumption. The average feed consumption (AFC) was determined by dividing the group feed consumption by the number of bird-days. The feed conversion ratio (FCR) was computed by dividing the AFC by ADG. At the end of the feeding trial, one sample broiler per treatment was dressed following three hours of feed withdrawal. The broilers were euthanized humanely by cervical dislocation method, after which the broilers were bled and defeathered. The dressing percentage was determined as the quotient of carcass weight and fasted live weight multiplied by 100. Furthermore, weight uniformity was computed by determining the number of broilers with weights within $\pm 10\%$ of the average weight of the group divided by the number of broilers weighed. Livability was computed by dividing the total number of broilers at the end of the study by the initial number of broilers and multiplying by 100. The mean sale value was computed as the product of the farm gate price per kilogram and the live weight. The cost of feed was computed as the AFC of the broilers (kg) multiplied by the cost per kilogram of the diet, while the mean income over feed cost (IOFC) was determined as the difference between the mean sale value of broilers and the mean cost of feed they consumed. For the microbial analysis,



three broilers were randomly selected per treatment for gut sampling on the 17th, 19th, 24th, 26th, and 32nd day of rearing. The broilers were euthanized humanely by cervical dislocation method. The intestines were laid out and cut, starting from the duodenum up to the colon right before the cloaca, and the ends were tied up with a sterilized thread. The gut samples were stored in air-tight plastic containers, labeled accordingly, and stored at -20°C. The samples were delivered immediately to the laboratory for microbial analysis.

Statistical analysis: Data were examined for normality and homogeneity using the Kolmogorov-Smirnov test, and after which were subjected to an Independent Samples *t*-test and presented as means $\pm$ standard deviation (STDEV), *t*-value, and the level of significance (*p*-value). Microbial counts were Log-transformed (Log_{10}) to normalize data distribution. Differences between means were assessed, and values of $p < .05$ were considered significant. All tests were done using the Statistical Tool for Agricultural Research (STAR) version 2.0.1 (IRRI, 2014).

RESULTS AND DISCUSSION

Growth performance: The mean effects on the growth performance of broilers of a combination of AML and probiotics compared with ZnB are presented in Table 1. The ADG of the broilers compared well, irrespective of the dietary additives in all periods, although numerically higher for the AML+P than its counterpart starting at 15 d. The AFC was also lower for the latter, which approached statistical significance at 0-14 d. Such differences in these parameters were reflected in lower FCR for the broilers given AML+P than those given ZnB, which reached significant differences, except at 25-32 d. These findings indicated that AML+P promoted the performance of broilers superior to ZnB, profoundly in terms of enhancing FCR. It was interesting to note that AML+P seemed to have influenced growth. In a related study by Saleh et al. (2021), AML supplementation (250 g/t) tended to increase gain in weight of broilers as well. FCR of broilers were significantly affected by AML+P supplementation at the first week ($t(18) = 3.58, p < .01$); second week ($t(18) = 2.82, p < .05$); and overall FCR ($t(18) = 2.88, p < .05$). On the other hand, Mustafa (2018) reported enhanced both ADG and FCR of broilers with 4 g AML/kg of diet. These data reflected the variability in responses to AML+P due to level of inclusion and possibly other factors employed in the conduct of the studies. On the other hand, an important finding in the present study was the enhancement in FCR of the broilers supplemented with AML+P, indicating enhancement of utilization and metabolism of nutrients. Such could have been the consequence of their additive or synergistic actions that promoted a healthy and functional gut. The antipathogenic role of AML (Szabo et al., 2023) and P (Naeem & Bourassa, 2025) could not be discounted as an important factor in this respect, as can be substantiated by the

non-detectable *C. perfringens* in the digesta of broilers presented in the succeeding section. It was plausible that the healthy status of the gut promoted more nutrients to be digested, absorbed, and metabolized efficiently for growth. AML is known for its antimicrobial activity, particularly against Gram-positive pathogens, which helps reduce microbial competition and inflammation in the gut (Szabo et al., 2023). This antimicrobial action likely created a more favorable intestinal environment for probiotic colonization and activity. Each probiotic species contributes distinct metabolic and immunological benefits: *B. subtilis* enhance enzymatic digestion and modulates immune responses (Al-Khalaifah, 2025), *Lactobacillus spp.* produce lactic acid and SCFAs that improve gut barrier function and nutrient absorption (Kumar et al., 2025), and *S. cerevisiae* support fiber fermentation and vitamin synthesis (Elhalis, 2024). Their combined application with AML likely amplified these effects, resulting in improved feed conversion and metabolic efficiency, aligning with established mechanisms described in the literature.

Table 1. Mean ($\pm$ STDEV) effect of ZnB and AML+P on the growth performance of broilers.

Parameters	ZnB	AML+P	<i>t</i> -value	<i>p</i> -value
ADG (g/day)				
0 - 14d	31.30 $\pm$ 0.74	31.31 $\pm$ 0.82	0.03	0.9772
15 - 24d	81.65 $\pm$ 3.99	83.66 $\pm$ 2.99	1.28	0.2167
25 - 32d	101.01 $\pm$ 6.95	102.60 $\pm$ 7.81	0.48	0.6351
0 - 32d	58.07 $\pm$ 3.12	59.27 $\pm$ 2.47	0.95	0.3536
AFC (g/day)				
0 - 14d	37.82 $\pm$ 0.59	36.94 $\pm$ 0.82	2.76	0.0130*
15 - 24d	115.43 $\pm$ 4.25	114.71 $\pm$ 2.68	0.46	0.6497
25 - 32d	161.22 $\pm$ 11.6	160.07 $\pm$ 5.15	0.29	0.7802
0 - 32d	83.18 $\pm$ 4.92	82.80 $\pm$ 3.77	0.20	0.8469
FCR (g/g)				
0 - 14d	1.21 $\pm$ 0.02	1.18 $\pm$ 0.01	3.58	0.0021**
15 - 24d	1.41 $\pm$ 0.04	1.37 $\pm$ 0.03	2.82	0.0114*
25 - 32d	1.60 $\pm$ 0.06	1.57 $\pm$ 0.08	0.96	0.3508
0 - 32d	1.43 $\pm$ 0.02	1.40 $\pm$ 0.03	2.88	0.0101*
Livability %	96.00 $\pm$ 4.22	97.60 $\pm$ 3.86	0.88	0.3880

* indicates *p*-value < 0.05; ** indicates *p*-value < 0.01

Carcass recovery: Table 2 shows the mean ALW, dressed weight, dressing percentage, and weight uniformity of broilers supplemented with either ZnB or AML+P. The ALW and carcass parameters were not affected by the supplements. This finding indicated that the deposition of fat and lean in the broilers was not influenced by the supplements. Amount and ratio of nutrients influence body composition and thus carcass yield (Toghyani et al., 2024). On the contrary, body weight uniformity tended to be higher for broilers supplemented with AML+P than those with ZnB (84.10% vs. 76.20%). The 7.90% higher body weight uniformity of broilers supplemented with AML+P reflected



more broilers in the population achieving optimal nutrition associated with this preparation.

Table 2. Mean ($\pm$ STDEV) effect of ZnB and AML+P on the carcass recovery of broilers.

Parameters	ZnB	AML+P	t-value	p-value
ALW (g)	2055.0 $\pm$ 67.04	2100.0 $\pm$ 63.77	1.54	0.1414
Dressed	1501.4 $\pm$ 54.21	1505.7 $\pm$ 40.93	0.20	0.8436
Weight (g)				
Dressing	73.60 $\pm$ 2.01	74.00 $\pm$ 1.76	0.47	0.6420
Percentage				
Weight	76.20 $\pm$ 6.70	84.10 $\pm$ 11.35	1.90	0.0741
Uniformity (%)				

Income over feed cost: The mean effect of ZnB and AML+P supplementation on the sale value per bird, cost of feed per bird, and income over feed cost is presented in Table 3. Statistical differences due to the supplements were found to be insignificant ($p>0.05$). It was notable, though, that the sale of broilers was 5.22% higher for the broilers supplemented with AML+P than for those with ZnB. Moreover, the cost of feed was lower by 0.74% for the former than for the latter. The combined effects of these resulted in 3.71% higher IOFC realized from broilers supplemented with AML+P. These data reflected the economic advantage of AML+P as a replacement for ZnB in the diet of broilers.

Table 3. Mean ($\pm$ STDEV) effect of ZnB and AML+P on the IOFC of broilers.

Parameters	ZnB	AML+P	t-value	p-value
Sale value, PhP ¹	192.68 $\pm$ 10.6	202.73 $\pm$ 6.71	0.77	0.4531
Cost of feed /bird, PhP ²	98.08 $\pm$ 3.55	97.35 $\pm$ 2.41	0.54	0.5959
IOFC ³	101.60 $\pm$ 9.13	105.37 $\pm$ 6.78	1.05	0.3078

¹Prevailing market price in the Philippines was PhP 100/kg liveweight

²Computed based on the amount of feed consumed per stage of growth and price per kg of the diet

³Sale value less cost of feed

Microbiota status: Table 4 presents the mean population of LAB and *C. perfringens* in the intestinal digesta of the broilers supplemented with either ZnB or AML+P at different measurement periods. The commensal LAB count was generally comparable for both broilers given ZnB and AML+P in all periods, except at 31 d, with significantly lower counts of those under the latter than the former ($p<0.01$). LAB count generally decreased with the age of the broilers. *C. perfringens* was not detectable in both groups at 17 d of age. However, *C. perfringens* was present in broilers supplemented with ZnB at 19, 24, 26 d, where log counts were the same (1.30 $\pm$ 0.00), and increased (1.48 $\pm$ 0.00) at 31 d. On the contrary, *C. perfringens* remained undetectable in broilers supplemented with AML+P in any period. The adverse effect

on LAB and the non-detectable population of *C. perfringens* in the digesta of broilers could have been due to the broad-spectrum antimicrobial activity of AML against both beneficial and pathogenic bacteria. Matsue et al. (2019) reported that lauric acid, to which AML belongs, has low antimicrobial activity against commensal LAB but high activity against pathogenic *Clostridium* and *Bacteroides*. Schlievert et al. (2018) also reported that AML was effective in inhibiting the vegetative cells and spores of aerobic *B. anthracis*, *B. subtilis*, and *B. cereus* and anaerobic *C. perfringens* and *C. difficile*. As an adjunct, the role of probiotics in terms of the microbiota status in the digesta of the broilers could not be discounted. Collado et al. (2010) stated that probiotics maintain healthy gut microflora through competition with pathogens for nutrients, preventing pathogens from adhering to the gut lining, and decreasing the activity of pathogenic bacterial enzymes. It was likely as well that the probiotics contributed to the increase in the beneficial bacterial population, enhancing such favorable effects of the AML+P on the gut. Overall, AML+P was superior to ZnB for modulating gut microbiota based on the aforementioned premises. The healthy gut of broilers resulting from the supplementation of AML+P played a major role in improving efficiency as discussed earlier.

Table 4. Mean ($\pm$ STDEV) effect of ZnB and AML+P on the intestinal microbiota of broilers.

Parameters	ZnB	AML+P	t-value	p-value
LAB				
17d	6.28 $\pm$ 1.08	5.72 $\pm$ 0.55	0.80	0.4678
19d	5.26 $\pm$ 0.17	4.33 $\pm$ 0.93	1.72	0.1613
24d	5.38 $\pm$ 0.49	5.77 $\pm$ 0.59	0.88	0.4283
26d	5.42 $\pm$ 0.44	5.77 $\pm$ 0.05	1.33	0.3124
31d	5.72 $\pm$ 0.08	5.04 $\pm$ 0.04	13.08	0.0002**
<i>C. perfringens</i>				
17d	ND	ND	-	-
19d	1.30 $\pm$ 0.00	ND	-	-
24d	1.30 $\pm$ 0.00	ND	-	-
26d	1.30 $\pm$ 0.00	ND	-	-
31d	1.48 $\pm$ 0.00	ND	-	-

Mean microbial counts are expressed as Log₁₀ CFU/g

* indicates p-value < 0.05; ** indicates p-value < 0.01

Conclusion: Dietary supplementation of AML+P improved the feed conversion efficiency of broilers and promoted a healthy gut based on the non-detectable *C. perfringens* in their intestinal digesta. Moreover, it is economical to supplement the broiler diet with AML+P. Thus, the combination of Alpha monolaurin and multispecies probiotic mix can be a potential replacement for Zinc Bacitracin in antibiotic-free poultry farming.

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Conflict of interest: The authors declare no conflict of interest.

Ethical statement: The Institutional Animal Care and Use Committee (IACUC) of Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, approved the animal care and use protocol of this research study and has issued a certificate of protocol review with reference number 20240903-01.

Availability of data and material: The data is available with the corresponding author which can be made available upon request

Consent for publication: All authors consented to the publication of this research article in JGIAS.

SDGs addressed: No poverty; Zero hunger; Good health and well-being; Responsible consumption and production; Life on land.

Policy referred: World Health Organization (WHO) guidelines; European Union (EU) Regulation; Philippine FDA & Department of Agriculture (DA) regulations; National Action Plan on Antimicrobial Resistance (NAP-AMR).

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